FACULDADE DE CIÊNCIAS DA NUTRIÇÃO E ALIMENTAÇÃO
UNIVERSIDADE DO PORTO

Controlo metabólico em doentes com Fenilcetonúria (PKU): impacto do aumento de fenilalanina para o teste de sobrecarga com BH4

Metabolic control in patients with Phenylketonuria (PKU): impact of phenylalanine titration for BH4 loading test

Alexandra Catarina de Sousa Barbosa

Orientado por: Júlio César Leite da Fonseca Rocha

Coorientado por: Nuno Pedro Garcia Fernandes Bento Borges

Trabalho de Investigação

Centro de Referência na área das Doenças Hereditárias do Metabolismo

Centro Hospitalar do Porto, E.P.E.

Centro de Genética Médica Doutor Jacinto de Magalhães

1.º Ciclo em Ciências da Nutrição

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Resumo

Introdução: Em Portugal, todos os doentes com PKU são submetidos a um teste de sobrecarga (TS) para avaliar a sua resposta à BH4. Antes do TS, a ingestão de fenilalanina (Fen)/proteína natural (PN) é aumentada para atingir valores de [Fen] no sangue > 480 µmol/L. Pretende-se verificar o impacto do aumento de Fen/PN no controlo metabólico pós-TS, particularmente nos não-respondedores.

Metodologia: 58 doentes PKU (4-34 anos; 19.6±8.2 anos; 50% mulheres; 48.3% PKU clássica, 51.7% PKU moderada) que completaram o TS em 2015 foram estudados em 4 períodos de estudo (PE). No PE1 (2010-2013) o tratamento foi exclusivamente dietético. No PE2 (2014) iniciou-se o aumento da ingestão de Fen/PN. Os TSs foram concluídos no PE3 (2015). No PE4 (2016) coexistiram doentes tratados exclusivamente com dieta (N=49) e doentes a tomar BH4 (N=9). Foram recolhidos dados de antropometria, ingestão proteica e controlo metabólico.

Resultados: A percentagem de doseamentos de Fen dentro do intervalo recomendado foi maior no PE1 vs. PE4 (64 [28-85] vs. 45 [0-66]; p<0,001). No PE1, 39,7% dos doentes tinham bom controlo metabólico, enquanto no PE4 este valor foi de apenas 22,4%. A mediana das diferenças da percentagem de valores dentro do intervalo recomendado entre o PE4 e o PE1 não foi estatisticamente diferente quando comparados os doentes tratados com BH4 vs. com dieta: -1 [-46;22] vs. -17 [-33;0]; p=0,408.

Conclusão: Apesar do controlo metabólico piorar com a idade, os resultados sugerem que um aumento temporário da ingestão de Fen/PN poderá afetar negativamente o controlo metabólico. São necessários mais estudos acerca do efeito do TS a longo prazo, revendo a duração e as estratégias utilizadas na preparação do mesmo.

Palavras Chave: *Fenilcetonúria; Teste de sobrecarga BH4; Controlo metabólico*

Abstract

Background: In Portugal, for PKU, all potential BH4 responders are identified using a loading test (LT). Phenylalanine (Phe)/natural protein (NP) intake is increased to elevate blood [Phe] > 480 µmol/L prior to LT. We aimed to verify the impact of Phe/NP titration on metabolic control post-LT in PKU patients, particularly in non-responders.

Patients and Methods: 58 PKU patients (4-34 y; 19.6 ± 8.2 y; 50% females; 48.3% classical PKU, 51.7% mild PKU) that completed LT in 2015 were studied in 4 different study periods (SP). At SP1 (2010-2013) patients were exclusively diet treated. Phe/NP titration was started during SP2 (2014). LTs were concluded in SP3 (2015). In SP4 (2016) patients were either exclusively diet treated (N=49) or BH4 treated (N=9). Anthropometry, protein ingestion and metabolic control data were collected and analyzed.

Results: Blood Phe measurements within target range (median %) was higher in SP1 vs. SP4 (64 [28-85] vs. 45 [0-66]; p<0,001). In SP1 there were 39.7% of patients under good metabolic control compared to 22.4% in SP4. The median of differences of blood Phe measurements within target range between SP4 and SP1 was not statistically different in BH4 treated vs. diet treated patients: -1 [-46;22] vs. -17 [-33;0]; p=0,408.

Conclusion: Although worsening blood [Phe] control may occur over time, our results suggest that transient Phe/NP titration may adversely affect long term blood [Phe] control. Studies examining the long-term effect of LT, including a review of length and strategy of preparation for LT are necessary.

Keywords: *Phenylketonuria; BH4 loading test; Metabolic control*

Índex

1. Introduction

Phenylketonuria (PKU) is an autosomal recessive inborn error of phenylalanine (Phe) metabolism, affecting 1:10000 newborn babies in Europe, caused by a phenylalanine hydroxylase (PAH) enzyme deficiency [1]. Therefore, the conversion of Phe (either from diet or endogenous catabolism) into tyrosine (Tyr) is compromised, resulting in increased blood [Phe]. Phe is a large neutral amino acid able to cross the blood brain barrier using L-amino acid transporter 1 (LAT1). High brain [Phe] leads to neuropsychological impairment through different mechanisms: (1) decrease in myelin formation in brain white matter; (2) inhibition in brain availability of Tyr (dopamine and norepinephrine precursor), tryptophan (serotonin precursor) and other large neutral amino acids except Phe, compromising protein and neurotransmitter synthesis; (3) inhibition of key enzymes involved in neurotransmitter synthesis and intermediary metabolism [2]. Thus, neonatal screening is essential to early identify PKU patients in order to promptly implement treatment, preventing future intellectual disability [1, 2]. Diet is the core of PKU treatment, involving a severe restriction of Phe and natural protein (NP) intake, replacement of non-Phe protein with a protein substitute (PS) and low-protein foods (including special low-protein foods) to satisfy energy needs [3, 4]. In Portugal, PS are available as either Phe-free amino-acid mixtures, glycomacropeptide-based PS and large neutral amino acids [5].

Maintaining life-long adherence to this restricted diet is challenging, especially in older children [6]. It is well established there is a deterioration of metabolic control over time, particularly in late adolescence and adulthood [6-9].

More recently, the administration of pharmacological doses of tetrahydrobiopterin (BH4), the PAH co-factor, available as a commercial formula of sapropterin dihydrochloride (Kuvan®), may help improve metabolic control, improving dietary Phe tolerance in a sub-set of patients with mild or moderate PKU [10-12]. BH4 responsiveness can be established either by genotype or with a loading test (LT) [1]. The PKU European guidelines suggest that in patients without two known disease null mutations or two known BH4 responsive mutations, a LT should be performed [1]. A 48h BH4 LT protocol has been used in Europe, analysing blood [Phe] before and after a single daily dose of sapropterin (20 mg/Kg/day) on two consecutive days [13, 14].

However, different methodologies in different countries are used to evaluate BH4 responsiveness [13, 14]. In Portugal, the Portuguese Society for Metabolic Disorders (SPDM) policy is to identify all potential BH4 responders using a LT. Phe and NP intakes are increased to elevate blood [Phe] > 480 µmol/L prior to LT and sometimes this procedure is challenging both for patients and professionals [15]. There are no studies describing the impact of a short-term increase in Phe and NP intake on long term blood [Phe], particularly in non-responders.

In a longitudinal, retrospective study, we aimed to examine the impact of Phe and NP titration on metabolic control post-LT in PKU patients.

2. Methods

2.1 Participants

In 2015, 66 patients with PKU completed a LT from the Reference Center of Inherited Metabolic Diseases of Centro Hospitalar do Porto. Eight patients were

excluded from the study: 5 were late diagnosed (late treated patients with inconsistent dietary compliance), 2 had insufficient clinical data and dietary records and 1 had Down Syndrome with severe neurological impairment affecting dietary management [16]. The final sample included 58 (N=58) early treated patients (4-34 years; 19.6 ± 8.2 years; 50% females). Disease severity was classified according to the neonatal blood [Phe], as stated at the Portuguese Consensus [17]: hyperphenylalaninemia (blood [Phe] < 6 mg/dL), mild PKU (blood [Phe] ≥ 6 mg/dL and ≤ 20 mg/dL) and classical PKU (blood [Phe] > 20 mg/dL). There was 30 mild PKU patients (51.7%) and 28 classical PKU patients (48.3%). The blood [Phe] target range was ≤ 6 or ≤ 8 mg/dL with patients aged < 12 or ≥ 12 years, respectively [17].

The final sample of patients was studied in 4 different study periods (SP). During the study period SP1 (2010-2013) all patients were on a low Phe diet. In SP2 (2014), the NP prescription was increased to define maximum Phe tolerance to establish a blood [Phe] > 480 µmol/L, according to SPDM protocol. In the SP3 (2015), the preparation for LT was continued and patients were gradually enrolled, while some potential BH4 responders had already started sapropterin treatment. In the SP4 (2016), patients were either exclusively diet treated (N=49) or under BH4 treatment (N=9).

2.2 Study Design

This is a longitudinal retrospective study, and data was collected from 2010 to 2016. Gender, birthdate, neonatal blood [Phe], disease severity, genotype, date of LT and sapropterin responsiveness were collected from electronic clinical records

of patients. Participants were identified by a code, preventing patient's identification. Data on anthropometry and nutritional intake were collected from clinical records from the final nutritional appointment of each SP. All blood Phe measurements done in SP1, SP2, SP3 and SP4, for each patient, were collected from the patient database.

2.3 Data Collection

2.3.1 Anthropometry

Weight and height were measured when patients were in light clothing only, without shoes and accessories. Seca® mechanic scale (measuring scale = 1 Kg) and a stadiometer (measuring scale = 1 mm) were used. Body mass index (BMI) was calculated as weight (Kg) / height^2 (m) and classified by World Health Organization criteria [18]. Anthro® and Anthro Plus® software were used to calculate BMI z-scores for patients aged between 0-5 years and 5-19 years, respectively. For patients aged 0-5 years, overweight was defined when BMI z-score was > 2 standard-deviations [19, 20]. In patients aged between 5-19 years, overweight was considered when BMI z-score was > 1 standard-deviation [20, 21].

2.3.2 Nutritional Intake

Dietary assessment was performed by a 24-hour recall. NP (g/Kg/day), protein equivalent (PE) from PS (g/Kg/day) and total protein (TP) (g/Kg/day) intakes were calculated.

2.3.3 Metabolic Control

Blood [Phe] was measured from blood-spots by tandem mass spectrometry. All patient blood Phe measurements in each of the 4 SP were calculated for median, mean and standard-deviation of blood [Phe]. Furthermore, for each period and

each patient, percentage of blood Phe measurements within target range was calculated. Whenever patients reached 12 years old (N=14), the upper target range was adjusted so results were correctly interpreted [17].

2.4 Ethical Statement

This study and its data collection were under the ethical approval consented by the Ethics Committee of Centro Hospitalar do Porto, on the18th of May 2015, to the investigation project *TNSPKU (Trends in Nutritional Status of patients with phenylketonuria),* with the reference *2015.101 (092-DEFI/087-CES).* Written informed consent was obtained from each patient or caregiver.

2.5 Statistical Analysis

IBM SPSS Statistics 24 for Windows was used for statistical analyses. Kolmogorov-Smirnov test was done to evaluate normal distribution of variables. Categorical variables were presented as absolute values or percentage, and continuous variables were presented as mean ± SDs or as medians [P_{25}-P_{75}], according to its distribution. Wilcoxon test and Mann-Whitney test were used to identify differences when non-normal distribution was found. The level of significance considered was $p<0.05$.

3. Results

Patient's characteristics are described in Table 1. No overweight or obesity was found in patients < 5 y, whereas the prevalence increased in adults (Table 2).

None of the patients who were overweight or obese in 2013 became normal weight in 2016 (data not shown).

Table 1. Gender, age and disease severity of patients studied.

Sample Size	N=58
Gender	Female: N=29 (50%) Male: N=29 (50%)
Age (at LT [a] - 2015)	19.6 ± 8.2 y (Min=4 y; Max=34 y) <19 y: N=24 (41.4%) ≥19 y: N=34 (58.6%)
Disease Severity	Mild PKU: N=30 (51.7%) Classical PKU: N=28 (48.3%)

[a] **LT – BH4 loading test**

Table 2. Anthropometric data of patients studied in SP1 vs. SP4.

Anthropometry	**SP1**			**SP4**		
	[0-5] y N = 3	]5-19] y N = 28	>19 y N = 27	[0-5] y N=0	]5-19] y N = 24	>19 y N = 34
BMI	-	-	22.8±4.6	-	-	23.9±4.7
BMI z-score	0.30±0.6	0.23±1.1	-	-	0.17±1.2	-
Overweight / Obesity	N = 0	N = 9	N = 6	-	N = 9	N = 11
Overweight / Obesity Prevalence (%)	25.9%			34.5%		

During the 4 study periods, NP intake (g/kg/day) remained similar, with a trend to a lower PE intake (g/kg/day), which is reflected in a lower TP intake in ST4 (Table 3).

Table 3. Protein intake in patients.

Protein intake	SP1	SP2	SP3	SP4
Natural protein intake (g/Kg/day)	0.58 ± 0.3	0.53 ± 0.3	0.50 ± 0.3	0.55 ± 0.3
Protein equivalent intake (g/Kg/day)	1.12 ± 0.4	0.95 ± 0.3	0.92 ± 0.3	0.84 ± 0.30
Total protein intake (g/Kg/day)	1.72 ± 0.4	1.49 ± 0.3	1.46 ± 0.4	1.41 ± 0.3

Median blood [Phe] was lower in SP1 than in SP4 (6.80 [4.70-10.30] vs. 7.91 [6.53-11.11]; p<0,001) (Table 4). The percentage of blood [Phe] within target range was higher in SP1 compared with SP4 (64% [28-85] vs. 45% [0-66]; p<0,001) (Table 4). In SP4, 16 patients (27.6%) did not have any blood Phe measurement within the target range compared with 6 (10.3%) patients in SP1. Changes in blood [Phe] control throughout the study are illustrated in Figure 1.

Table 4. Metabolic control of patients during the 4 periods of the study.

Metabolic Control	SP1	SP 2	SP 3	SP 4
Median [Phe] [a] (mg/dL)	6.80 P_{25} = 4.70 P_{75} = 10.30	7.98 P_{25} = 5.85 P_{75} = 10.58	8.20 P_{25} = 6.80 P_{75} = 10.25	7.91 P_{25} = 6.53 P_{75} = 11.11
Phe measurements within target range (%)	64 P_{25} = 28 P_{75} = 85	47 P_{25} = 7 P_{75} = 76	38 P_{25} = 18 P_{75} = 62	45 P_{25} = 0 P_{75} = 66

[a] Phe - Phenylalanine;
P25 - 25th percentile;
P75 - 75th percentile.

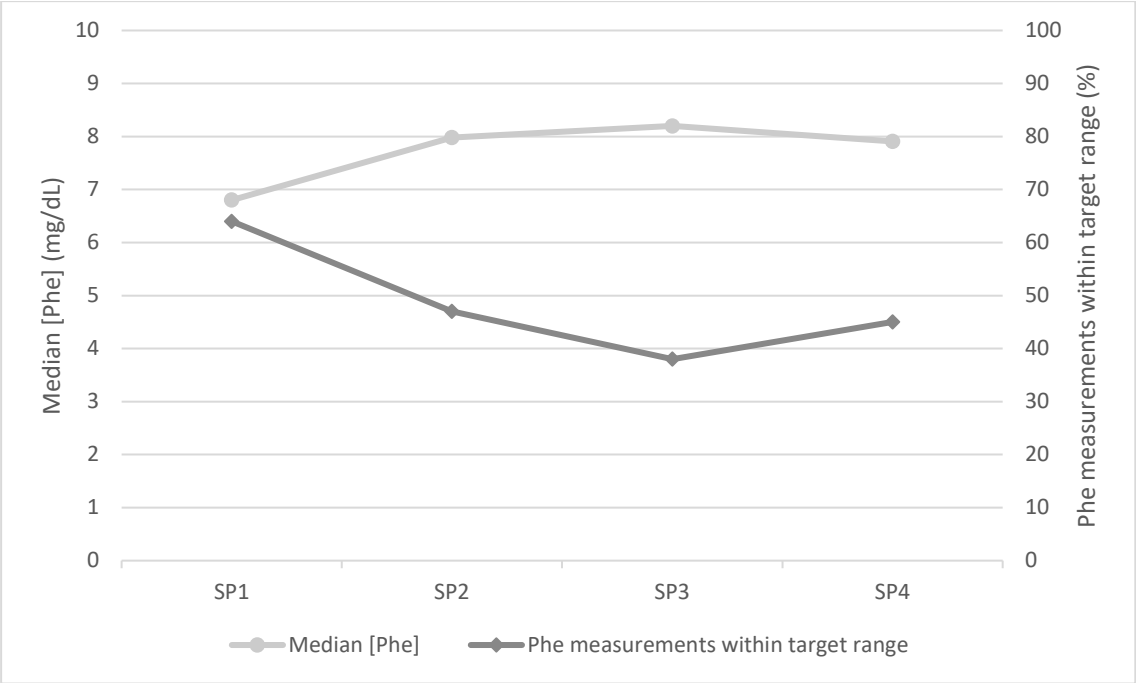

Figure 1. Evolution of metabolic control of the 58 patients studied.

In SP1 there were almost twice as many patients in good metabolic control (39.7%) compared to SP4 (22.4%) (Table 5). Table 6 presents data on percentages of blood Phe measurements within target range at SP1 and SP4, for patients on sapropterin treatment and diet treatment only. In these sub-groups, the median of differences between SP1 and SP4 was -1 [-46;22] and -17 [-33;0], not reaching statistical significance (p=0,408) (Table 6).

Table 5. Metabolic Control Quality.

Metabolic Control (% of patients per class)	**SP1**		**SP4**	
	<75% *"Bad control"*	≥75% *"Good control"*	<75% *"Bad control"*	≥75% *"Good control"*
	60.3	39.7	77.6	22.4

Phe measurements within target range ≥75% - "Good control"
Phe measurements within target range <75% - "Bad control"

Table 6. Metabolic Control.

Metabolic Control (% of Phe measurements within target range)			
On BH4 Treatment in 2016 (N=9)[a]		**Without BH4 Treatment in 2016 (N=49)**	
SP1	**SP4**	**SP1**	**SP4**
90 P_{25} = 65 P_{75} = 97	83 P_{25} = 48 P_{75} = 91	58 P_{25} = 21 P_{75} = 80	41 P_{25} = 0 P_{75} = 57
Median of differences = -1 [-46;22]		Median of differences = -17 [-33;0]	

[a] Some patients have responded to LT but only initiated medication during/after 2016 or stopped medication (N=6). This table only represents patients on medication in 2016.

4. Discussion

The most important finding of this study is the suggestion that patients who received a sapropterin LT, with a temporary increase in NP pre-test, had a worse Phe control one year post test, which later saw a trend towards a slight improvement. Both the percentage of blood Phe measurements within target range at SP1 compared with SP4, and the contrast between percentage of patients under good metabolic in SP1 and SP4, support our conclusions.

We recognise that many factors may have led to deterioration in blood [Phe] control in addition to sapropterin LT. Diet treatment for PKU patients is extremely restrictive and PS have a strong taste and odor, so adherence is challenging [7, 8]. After the age of 10 years, it is well recognised that dietary adherence is challenging, associated with dietary management transition to self-care, increasing

socialisation and hunger [6, 7]. Accordingly, a progressive deterioration in metabolic control with age, particularly in adolescents and younger adults is established [1, 6, 8]. During adolescence and adulthood, there is a relaxation of the diet: pleasure of food becomes more important, there are time constraints and stress associated with food preparation, and sometimes it is difficult to comprise restrictions of these patients with their lifestyle [9].

Considering that during the 4 SP the cohort of patients increased in age, it seemed more appropriate to analyse the percentage of blood Phe levels within target range rather than median blood levels, as target ranges are already age-adjusted [17].

The choice of foods for increasing NP intake for the Phe titration may have affected the results. Increasing Phe intake using milk sources together with PS was not practical in older patients as previously recommended [15]. In many adult PKU patients a liquid PS was prescribed, and it was not possible to add other NP sources to it. In addition, the known milder spectrum of the disease usually found in Portugal also influenced the practical strategies used to increase diet Phe intake [22]. It was verified high Phe tolerance in many patients, so regular foods with a high protein content was given for the temporary increase in Phe intake, e.g., regular bread and pasta, milk, cheese and yogurt.

Analysing the SP4 in particular, it seems that the degree of metabolic control deterioration was greater in the group without BH4 treatment compared with BH4 treated patients, suggesting a negative effect of the temporary exposure to the additional Phe prior LT. While some patients were afraid to introduce high protein foods into their daily routine, others enjoyed their taste [23]. We cannot rule out the psychological consequences on feeding behavior of giving additional NP and then

withdrawing it again in non-responders. The difficulty and disappointment in returning to their previous restricted diet should not be underestimated in non-responders [15].

In the process of Phe titration, PS prescription was maintained during preparation for LT. However, considering the weight variations throughout the study (Table 2), stabilizing PS prescription resulted in a lower TP intake interpreted in terms of g/kg. Also the observed weight changes may have justified an increase PE prescription, in order to theoretically prevent any negative effect in terms of metabolic control.

Our study has several limitations. First, there was no opportunity to identify the exact starting date of the LT preparation phase, because some patients already had blood [Phe] high enough to perform the test with no need to modify their diet. In practice, if the precise length of NP titration process was possible to be determined, a clearer impact could be seen. For the same reason, we were unable to describe the precise Phe/NP increase for each patient, by how much and by which foods, during preparation for LT. Moreover, this was a retrospective and non-controlled study that analyzed metabolic control in a group of PKU patients under follow-up and proposed for BH4-LT. Also, it was not possible to have a control group. Although we have used the percentage of blood Phe measurements within target range, which is already age-adjusted, our group had a wide age range which may have influenced compliance and metabolic control. Finally, in SP4, the comparison between BH4 treated and diet treated patients should be analyzed carefully due to the small group of patients under drug treatment. However, this is the first study evaluating the effect of the LT procedures on

metabolic control, involving just patients from one reference treatment centre and collecting data only from the year following the LT.

5. Conclusion

In conclusion, although a deterioration of blood [Phe] control may occur over time, our results suggest that a transient Phe and NP titration may further adversely affect metabolic control, particularly in non-responders. Even though the use of sapropterin has been described as a good co-adjuvant for BH4-responders, there are many different protocols for assessing BH4 responsiveness. A multicentre and controlled study would be helpful to examine the long-term effect of different methodologies on metabolic control, including a review of length and strategy of preparation for the LT and the assessment of its relevance.

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